

## Ras Signaling Pathway Proteins as Therapeutic Targets

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**Abstract:** Ras is a 21 kDa membrane-localized G protein that is coupled to receptor and non-receptor tyrosine kinase activation of downstream cytoplasmic and nuclear events. Mutated ras genes are common, and occur in a wide variety of human malignancies. These activating mutations result in constitutive signaling, thereby stimulating cell proliferation and inhibiting apoptosis. Preclinically, inhibitors of ras signaling revert ras-dependent cellular transformation, and cause regression of ras-dependent rodent tumor xenografts. The ras signaling pathway has therefore attracted considerable attention as a target for anticancer therapy. In this review, novel therapeutic approaches based on the inhibition of ras-mediated signaling, are described. The discussion will be limited to inhibitors which are currently in human clinical trials, and include inhibitors of ras processing, inhibitors of ras protein synthesis and inhibitors of downstream ras effectors.



### INTRODUCTION

A critical property of the malignant phenotype is dysregulated cell signaling and proliferation which commonly occurs through over-expression or mutation of proto-oncogenes. One such commonly mutated proto-oncogene is ras, which functions as a molecular switch in a large network of intracellular signaling pathways, mainly controlling the differentiation or proliferation of cells. Mutated ras genes encode constitutively activated proteins, which play a critical role in carcinogenesis. Ras mutations have been identified in approximately 30% of all human cancers, thus explaining the importance of this monomeric G protein as a target for the development of anti-cancer therapeutic and chemopreventive approaches.

### The Ras Superfamily

Ras belongs to a large superfamily of guanine nucleotide triphosphatases (GTPases) that regulate multiple cellular processes [1,2]. At least 20 members of this family, which share

approximately 30% sequence identity, are known in mammalian cells. These include M-Ras/R-Ras3, R-Ras, TC21/R-Ras2, Rap1A, -1B, -2A, and -2B, Ral A and B, Rit, Rin, Rheb, Rhes, B-Ras 1 and 2, as well as the classic Ras proteins [1]. The ras genes encode four highly related proteins H-ras, N-ras and K-ras, which comprises two splice variants (Kras4A- and Kras4B-) [3]. Each monomeric ras protein consists of approximately 190 highly conserved amino acid residues. Most of the differences between these proteins occur near the C-terminal hyper-variable domain of about 25 amino acids. This variable domain is thought to account for the different biologic functions of the ras protein isoforms. Signaling through ras proteins results in protein synthesis and regulation of cell survival, proliferation and differentiation.

The rho subfamily plays a role in regulation of the actin cytoskeleton, the rab subfamily is involved in membrane trafficking, the ARF subfamily mediates vesicle trafficking, and the Ran subfamily is involved in nuclear transport [1,2].

### Post-Translational Modification of Ras

Ras proteins are synthesized as small, hydrophilic molecules, which require addition of carbon residues to render them lipophilic, before anchoring into cell membranes where they function in cell signaling. This "post-translational

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modification" involves prenylation (addition of 15- or 20-carbon residues), proteolysis, carboxymethylation and palmitoylation [4] (Fig 1). Prenylation is catalyzed by three enzymes. The first enzyme, protein farnesyl transferase (FT) transfers a farnesyl group from farnesylpyrophosphate (FPP) to the terminal cysteine at the carboxyl end of target proteins. This enzyme recognizes a specific "CAAX" motif, where "C" represents cysteine, "A" represents an aliphatic amino acid (leucine, isoleucine or valine) and "X" is methionine, serine leucine, or glutamine. The second enzyme, protein geranylgeranyl transferase type I (GGT-I), transfers a geranylgeranyl group from geranylgeranyl pyrophosphate (GGPP), also to CAAX-containing proteins. The terminal "X" of the CAAX box determines whether farnesylation or geranylgeranylation occurs [5,6]. The third enzyme, protein geranylgeranyl

transferase type II (GGT-II) transfers geranylgeranyl groups from GGPP to proteins containing two carboxy-terminal cysteine (CXC) residues [7,8].

Farnesylation is the first, and rate-determining step in the post-translational modification of ras proteins (Fig "1") After farnesylation, ras proteins undergo two additional steps, proteolytic cleavage of the terminal tripeptide, "AAX" followed by carboxymethylation [9 and Fig 1]. Finally, palmitoylation of SH groups of the terminal cysteine residues occur prior to membrane anchorage. Palmitoylation is reversible, and occurs only for ras proteins with a cysteine residue upstream of the CAAX motif, ( H-Ras, N-Ras, and K-Ras4A ), whereas the other CAAX-triggered events are irreversible [10,11,12].

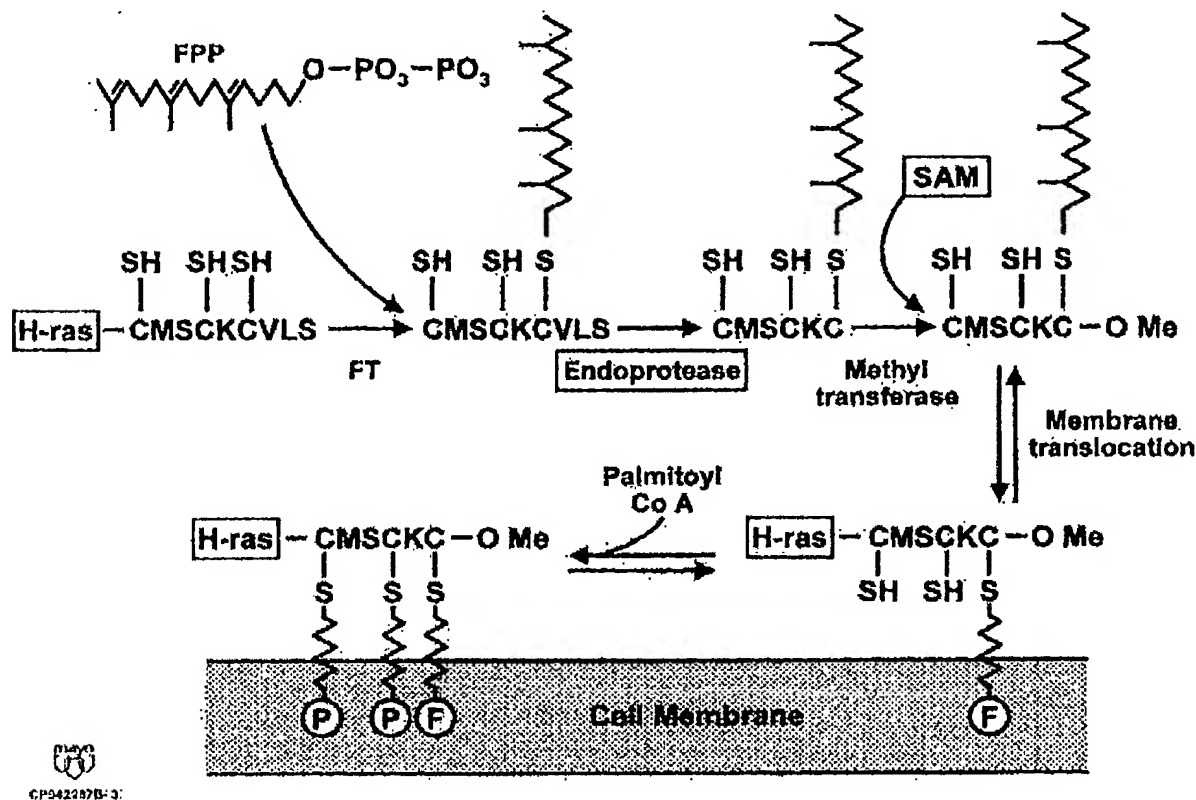


Fig. (1). Simplified scheme of the post-translational processing of H-ras, catalyzed by farnesyltransferase (FT). A farnesyl group (F) is transferred from FPP to the thiol group of the cysteine residue of CVLS (CAAX motif). The terminal tripeptide is cleaved by an endoprotease. Palmitoylation of C-terminal cysteine residues occurs before membrane localization. Reproduced with permission from Adjei AA. *Drugs of the Future* 25 (10):1069-1079, 2000. copyright Prous Science.

K-RasB is not palmitoylated, but possesses a cluster of lysine-rich sequences that may be responsible for increased affinity with the cell membrane, through electrostatic interactions with acidic phospholipids and other negatively charged groups on the inner membrane surface [13].

### The Ras Signaling Pathway

The function and biology of ras proteins have been comprehensively reviewed recently [14-17]. Membrane-localized ras cycles between the quiescent GDP-bound and the activated GTP-bound forms. After external stimulation through binding of ligands such as epidermal growth factor to their receptors, ras is activated through dissociation of GDP and binding of GTP [18].

This activation is catalyzed by Guanine Nucleotide Exchange Factors (GEFs) which include SOS, cdc25, and C3G [19]. Return to the quiescent state occurs through stimulation of the low intrinsic GTPase activity of ras by GTPase Activating Proteins (GAPs), which include neurofibromin and p120-GAP [20].

Active GTP – bound ras exerts its biological effects by activating several effectors that turn on downstream pathways.

### Ras Effectors

Several cytoplasmic targets of ras have been described. The specific involvement of a few of these effectors in ras function is unknown. These

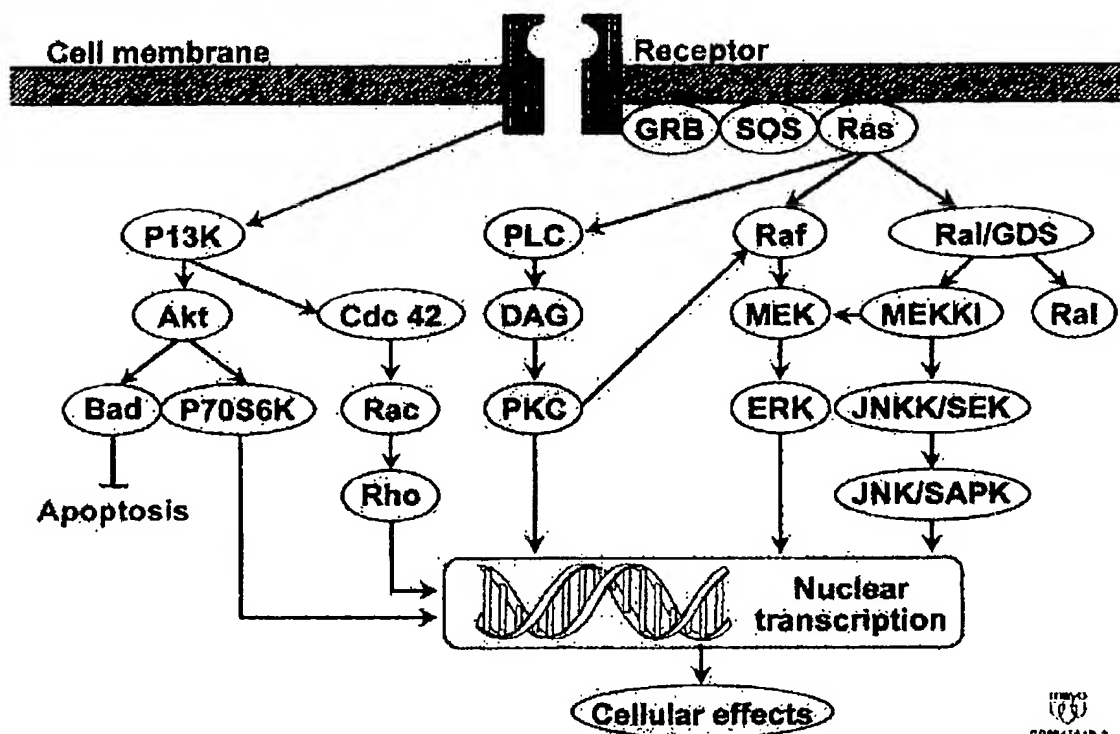


Fig. (2). Ras signaling and its effector pathways. DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; JNK, Jun amino terminal kinase; MEK, mitogen-activated ERK kinase; PI3K, phosphoinositide-3-kinase; PLC, phospholipase C; PKC, protein kinase C; SAPK, stress-activated protein kinase. The ras-MEK-ERK pathway is an example of a typical MAP kinase signaling module. Activated MAPK (ERK) phosphorylates and activates various transcription factors in the nucleus, which control cellular responses.

include protein kinase C  $\xi$  [21], Rin [22], and Af-6 [23].

The raf-MEK-ERK pathway is the most well characterized downstream effector system for ras. The soluble serine/threonine kinases B-Raf and c-Raf are recruited to the plasma membrane and activated by Ras-GTP through a poorly understood mechanism [24-40]. Localization of raf to the plasma membrane is essential for its activation. Thus, fusion of raf to the C-terminal membrane-localization signal of K-ras leads to its constitutive localization to the plasma membrane and bypasses the need for ras [26,27]. Activated raf triggers the activation of a cascade of protein kinases. MAP kinase or extracellular signal-regulated kinase (ERK1/ERK2) is activated by a MAP/ERK kinase (MEK, MAPKK), which is directly activated by Raf. ERK activation results in phosphorylation and activation of transcription factors such as *c-Jun*, *c-Myc* and *c-Fos*, resulting in the switching on of a number of genes associated with proliferation [41] (Fig 2). The critical effector function of raf is supported by several data. First, dominant-negative mutants of raf can impair ras-transforming activity [36,37]. Second, constitutively activated forms of raf possess transforming activity comparable to that of ras [37,38], and are themselves sufficient to transform some murine cells [39-41].

Another ras effector is the catalytic subunit of phosphatidylinositol 3-OH kinase (PI3K) [42]; this pathway leads to the activation of the protein kinase Akt [43,44], as well as the activation of Ras-related proteins of the Rho/Rac/Cdc42 family. Akt targets survival and death factors such as the pro-apoptotic proteins Bad [45] and caspase 9 [46] which are downregulated by Akt, and the kinase mammalian target of rapamycin (mTOR), whose downstream targets p70 S6 kinase and p115-1 are important in cell cycle progression [47,48] (Fig 2). The rho/rac/Cdc42 family is involved in controlling the polymerization state of the actin cytoskeleton, cell adhesion, and gene transcription [49,50]. Ras is also able to activate the related GTPase Ral through RalGEFs that are direct effectors of Ras [51-54]. Three such proteins, RalGDS [55], RGL [56], and Rlf [57] have been extensively characterized, and the isolation of another member of this family has been recently reported [58]. Recent data indicate that in most cellular systems, the complementary action

of at least two of these three pathways (Raf, PI3K, and RalGEFs) is necessary for Ras to transform murine fibroblasts in culture [50,52, 59-61].

The serine-threonine kinase MEKK1 is involved in the stress-response pathway, whose downstream targets include the MAP kinases c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK) [62,63]. The MEKK1/JNK/SAPK cascade induces apoptosis under certain circumstances [36]. MEKK1 can be activated by ras-GTP through the Ral/Cdc42 pathway [64]. Although the primary target of MEKK1 is JNK, recent evidence suggests that MEKK1 can activate the MEK/ERK pathway independent of raf-1 (Fig 2). Thus, JNK may be the target of ras in an alternate pathway, and not the MAP kinase pathway but co-operating with it [65].

## CLINICAL STUDIES OF INHIBITORS OF RAS SIGNALING

Because of the high percentage of human tumors harboring oncogenic *ras* mutants, and the key role played by such mutants in maintaining the malignant phenotype, interrupting the ras signaling pathway is an obvious focus of new drug development efforts. The major approaches that have yielded compounds currently in the clinic are:

- i) inhibition of ras protein expression
- ii) inhibition of ras processing
- iii) inhibition of downstream effectors of ras function.

## INHIBITION OF RAS PROTEIN EXPRESSION

### Antisense Oligodeoxynucleotides

Antisense oligonucleotides are short strands of chemically modified DNA or DNA - RNA hybrids, designed to regulate gene expression by binding through Watson-Crick base hybridization to a specific mRNA "sense" sequence. This results in blocking of the translation of the RNA message to a specific protein. Several mechanisms may account for the inhibition of gene expression after

Table 1. Inhibitors of ras Signaling Pathway Proteins

Target	Class/Agent	Phase of Development
H-ras mRNA	Antisense oligo-nucleotide ISIS 2503 <sup>73-75</sup>	Phase II
c-raf kinase mRNA	ISIS 5132 <sup>104</sup>	Phase II
farnesyl protein transferase	Small molecule inhibitor R115777 <sup>84-89</sup> SCH66336 <sup>95-99</sup> BMS214662 <sup>102</sup>	Phase III Phase III Phase II
c-raf kinase activity	Small molecule inhibitor	Phase I
MEK kinase activity	PD184322 <sup>106</sup>	Phase I
mTOR (inhibits p70 <sup>s6k</sup> and PIAS-1)	CCI-779 <sup>118, 119</sup>	Phase II
IISP90	17-AAG <sup>121</sup>	Phase I

• Superscripts are reference numbers.

• Small molecule raf-kinase inhibitors are in phase I trials, but information is not in the public domain.

antisense oligonucleotides bind to their target mRNA. The most widely characterized mechanism is the ribonuclease H (Rnase H)-mediated degradation of the mRNA strand [66]. The first demonstration of the therapeutic potential of antisense oligonucleotides was by Zamecnik and Stephenson in 1978. They utilized a short strand of synthetic oligodeoxynucleotide complementary to a specific mRNA sequence of Rous sarcoma virus to inhibit viral replication [67]. First generation antisense oligonucleotides in clinical use have one of the phosphate non-bridging oxygens of the internucleotide phosphodiester linkages replaced with sulfur. These phosphothiorate molecules are negatively charged and resistant to degradation by nucleases.

### K-Ras Antisense Oligonucleotides

K-ras antisense approaches have utilized large constructs incorporated into plasmids or viral vectors. For example, a 2-kilobase K-ras antisense sequence incorporated into an adenoviral vector generated K-ras antisense RNA, and reduced K-ras protein levels, inhibiting the growth of the H460A NSCLC cell line in culture [68]. This K-ras viral construct was successfully administered intratracheally to nude mice bearing implanted human lung cancers. Significant activity was observed with 87% of treated mice being tumor-free compared to 10% of control mice [68,69]. A different K-ras antisense RNA generated through liposomal transfection of a plasmid construct, has

demonstrated inhibition of human pancreatic cancer cells bearing the K-ras mutation, *in vitro*. No activity was observed in cell lines with wild-type ras. These plasmids were effective *in vivo*, when they were injected into the peritoneum of rodents bearing pancreatic cancer xenografts [70]. A drawback of the K-ras antisense strategies is the inability to deliver intact antisense RNA agents into tumor cells systemically. Direct intra-tumoral injection or regional delivery, have to be utilized, limiting the use of this approach in systemic metastatic disease.

### H-Ras Antisense Oligonucleotides

In contrast to the large constructs used in K-ras antisense therapy, H-ras antisense approaches have used phosphorothioate oligodeoxynucleotides whose distribution in rodent tissues after intravenous administration, presumably by endocytosis, has been demonstrated [71]. This allows for their systemic administration. ISIS 2503 is a 20-mer phosphothiorate oligodeoxynucleotides (5' TCCGTCATCGCTCCTCAGGG-3') which binds to a sequence in the translation initiation region of human mRNA for H-ras, with subsequent degradation of the hybridized mRNA by Rnase H. Preclinically, ISIS 2503 discriminates between H-ras and mRNA of K-ras and N-ras [72].

Two single-agent trials of ISIS 2503 have been reported. In the first trial, ISIS 2503 was

administered as a 14-day intravenous infusion followed by a 7-day rest period. Toxicities were mild to modest (National Cancer Institute Common Toxicity Criteria (NCI CTC) grades 1-2) and comprised transient fever, fatigue, thrombocytopenia, and nausea. Four out of twenty three patients had disease stabilization through 4-10 cycles of therapy. Maximum tolerated dose was not reached, but the dose of 6 mg/kg/day was defined for phase II studies based on preclinical modelling [73].

In the second study, ISIS 2503 was administered as a once weekly 24-hour infusion. The recommended phase II dose on this study was 18 mg/kg/day. In addition to the toxicities outlined above, myalgias, chills, thrombocytopenia, and microangiopathic hemolytic anemia were observed [74].

Based on a better tolerability profile, the 14-day infusion schedule was chosen for further development. Phase 2 trials are ongoing in breast, colon, non-small cell lung, and pancreatic cancers.

A phase I trial of a combination of ISIS 2503 and gemcitabine has been reported in abstract form [75]. Gemcitabine was administered at a fixed dose of 1000 mg/m<sup>2</sup> on days 1 and 8, with cycles repeated every 21 days. ISIS 2503 was administered as a continuous 14-day infusion at either 4 or 6 mg/kg/day. No dose-limiting toxicities were observed. Apart from 3 patients with grade 3 thrombocytopenia, all toxicities were mild to moderate, and included neutropenia, fever, nausea and vomiting, diarrhea and stomatitis. One patient with breast cancer, out of nineteen evaluable patients, had a 45% regression of her disease. This has been maintained for more than a year. Five patients had prolonged disease stabilization. This regimen (with an ISIS 2503 dose of 6 mg/kg/day) is being tested in phase II trials in pancreatic carcinoma.

## INHIBITION OF RAS AND/OR OTHER G-PROTEIN PROCESSING

### Farnesyltransferase Inhibitors

Because farnesylation is critical for ras maturation and function, farnesyltransferase inhibitors (FTIs) were rationally designed as a specific and sensitive inhibitor of ras-mediated

cellular proliferation [76]. These FTIs clearly inhibit ras farnesylation and cause regression of ras-transfected tumors in rodents. However, it has become clear in recent years that the critical target of FTIs may not be ras proteins, or may include other proteins in addition to ras. First, after FT inhibition, K-ras and N-ras proteins can be alternatively prenylated by geranylgeranylation [77]. These geranylated ras proteins are capable of inducing malignant transformation when over-expressed in cells. In spite of this, FTIs are active in vitro and in vivo in cells harboring activated K-ras mutations [78]. In addition, several types with ras mutation are sensitive to FTIs in vivo and in vitro. Such cells bearing wild type ras genes are in general more sensitive to inhibition by FTIs [79]. Currently 300 proteins possessing a "CAAX" box that can potentially be farnesylated have been identified in the EST database [80]. Up to twenty of these proteins, including, rho, lamins A and B, transducin, CENP-E and F, rhodopsin kinase and P and F have been well-characterized [81].

Prevailing hypotheses implicate the G-protein rho B, which regulates cytoskeletal organization, or a putative protein associated with the PI3-K/AKT survival pathway, as a critical target of the FTIs. A third possibility is that the cytotoxicity of FTIs may be due to inhibition of farnesylation of several critical proteins, including some or all of the ras isoforms.

FTIs are the first group of rationally synthesized agents designed to target oncogenic ras, to enter clinical trials. The biology, development and structural diversity of this novel class of anticancer agents have been reviewed in detail [81-83].

Three agents in this class are in phase I – phase III trials that have been reported.

### R115777

R115777, an orally bioavailable methylquinolone peptidomimetic FT inhibitor, was the first agent in this class to undergo clinical testing. The antitumor effects of this compound have been elucidated in cultured cells and xenografts in an elegant study by End *et al.* [79]. Using isolated human FT, they demonstrated that R115777 competitively inhibited the farnesylation of lamin B and KrasB peptides with IC<sub>50</sub>s of 0.86  $\mu$ m and 7.9  $\mu$ m respectively. Seventy-five percent of a

panel of 53 human cell lines were sensitive to R115777. Sensitive cell lines had a wild type gene or H-ras or N-ras mutations. Cell lines with mutant K-ras genes were relatively resistant. Surprisingly, in responding rodent-bearing xenografts, histological examination revealed a heterogeneity of responses to R115777. In LoVo human colon carcinoma tumors, an anti-angiogenic response was obtained. The responses in CAPAN-2 pancreatic cancer and C32 melanoma were an antiproliferative effect and apoptotic effect, respectively. These results indicate that the cellular effects of R115777, and presumably other PTTs is complex. However, the composite of the above effects is consistent with the ability of these agents to reverse the malignant phenotype of tumors, since this phenotype incorporates the properties of angiogenesis, proliferation and survival (resistance to apoptosis).

#### **Phase I Study of R115777**

Four phase I studies of R115777 have been reported. Three different schedules were tested in patients with solid tumors: i) oral administration twice daily for 5 days followed by 9 days of rest. ii) a 21-day oral twice-daily dosing schedule repeated on a 28-day cycle. iii) a continuous twice daily oral dosing schedule. The toxicity profile of R115777 was different in the different schedules. In the first schedule, the common toxicities were nausea, vomiting, headache, fatigue, anemia, and hypotension. In the prolonged administration schedules, myelosuppression was common. GI side effects were rare, and hypotension was not seen. Neuromotor and neurosensory deficits were seen in the continuous administration schedule, but not on the schedule with a one-week break in between cycles. Clinical activity was evidenced by a partial response in NSCLC and several patients with prolonged disease stabilization [84-86].

One phase I study has been completed in patients with refractory leukemia, with a 30 % response rate. Neutropenia was also the major toxicity seen in this study. No neurologic toxicities were seen, and GI toxicities were minimal [87].

#### **Phase II Study of R115777**

The first preliminary results of a phase II study with an PTT, has been reported for R115777 in patients with metastatic breast cancer who have

received one hormonal or chemotherapy treatment regimen. Twenty-seven evaluable patients received a continuous oral dose of 300 mg twice daily. The first 6 patients received a dose of 400 mg, which was later reduced to 300 mg because of severe neutropenia, which occurred by day 26. The most common toxicity was severe neutropenia (National Cancer Institute Common toxicity criteria [CTC] grades 3 and 4) which occurred in 26% of patients after a median of 32 days on treatment. Neutrophils recovered in 1 to 2 weeks in all patients. CTC grade 3 thrombocytopenia occurred in 11% of patients. Non-hematologic toxicities included CTC grade 2/3 peripheral neuropathy in 26% of patients after a median of 10 weeks, fatigue in 26% of patients, diarrhea and skin rash in 11% of patients each. Confirmed partial responses in soft tissue disease were seen in 12% of patients. Another 35% of patients had disease stability for at least 3 months [88].

Currently, other single-agent phase II studies are ongoing in a variety of tumor types.

#### **Phase I Study of R115777 in Combination with Gemcitabine**

A phase I combination study of R115777 and gemcitabine has been completed and reported in abstract form. The gemcitabine dose was fixed at 1000 mg/m<sup>2</sup> and administered on d1, d8, d15 out of every 28 days. Escalating doses of R115777 were administered on a continuous schedule. The most common and dose-limiting toxicity was neutropenia. Other toxicities were thrombocytopenia, nausea, vomiting, diarrhea and fatigue. Clinical activity was documented. The recommended phase II dose was 200 mg of R115777 with 1000 mg/m<sup>2</sup> of gemcitabine [89]. Combination studies with a variety of other chemotherapy agents including capecitabine, the taxanes, irinotecan, herceptin and gemcitabine/cisplatin are ongoing.

#### **Phase III Studies of R115777**

Two phase III studies of R115777 in pancreatic and colorectal carcinoma are nearing their accrual goals. Interim results are awaited with interest.

#### **SCH66336**

SCH66336 is a novel nonpeptidic tricyclic FT inhibitor, competing with the protein substrate for



binding to FT [90-92], and was introduced into clinical trials shortly after R115777. SCH66336 prevents the membrane association of H-ras, but not K- or N-ras. However, it reverts the anchorage-independent growth of human tumor cell lines harboring H-ras, K-ras, and N-ras mutations, in soft agar [93]. These findings support results of studies with other FTIs, which indicate that blocking farnesylation of proteins besides ras may be responsible for the antiproliferative effects of these agents [16, 81-83]. SCH 66336 alters the cell cycle distribution of sensitive human tumor cells in two distinct ways. Most sensitive cell lines accumulate in the G2/M phase after treatment with SCH66336, but those with an activated H-ras accumulate in G1 phase. The biological effects induced by SCH66336 in cells with an activated H-ras may therefore be distinct from other sensitive cells [94]. Further studies have indicated that cells with wild-type p53 are especially sensitive to SCH66336. In addition, farnesylation of the centromeric protein CENP-E is inhibited by SCH66336 [80]. This inhibition alters the microtubule-centromere interaction during mitosis and results in the accumulation of cells prior to metaphase. These results may explain some of the cell cycle effects of SCH66336, and presumably, other FTIs, and suggest that the centromeric proteins may contribute to the cytotoxicity of the FTIs.

#### Phase I Study of SCH66336

Three phase I studies in which SCH66336 was administered orally twice-daily for 7 days out of every 21 days, 14 days out of every 28 days, and continuously, have been reported. On the 7-day schedule, gastrointestinal toxicity (nausea, vomiting, diarrhea) and fatigue were common and dose-limiting. On the 14-day schedule, major toxicities were nausea, diarrhea and malaise. The most common and dose-limiting toxicities on the continuous administration schedule were neutropenia and thrombocytopenia. Other toxicities were diarrhea, anorexia, fatigue and weight loss. Clinical activity evidenced by a partial response in NSCLC and prolonged disease stabilisation have also been seen [95-97].

#### Phase I Combination of SCH66336 and Gemcitabine

A phase I combination study of continuous oral administration of SCH66336 with gemcitabine

administered on days 1, 8, 15 every 28 days has been reported in abstract form. Toxicities were nausea, vomiting, diarrhea and mild myelosuppression. The recommended doses for phase II testing were either SCH 66336 given at doses of 150 mg in the morning and 100 mg in the evening with 1000 mg/m<sup>2</sup> of gemcitabine, or a SCH 66336 dose of 200 mg twice daily with 600 mg/m<sup>2</sup> of gemcitabine [98]. Two partial responses were documented in pancreatic cancer, and one minor response each was documented in pancreatic cancer and pleural mesothelioma.

#### Phase I Study of SCH 66336 and Paclitaxel

A combination study of SCH66336 and paclitaxel has been reported in abstract form. The recommended phase II dose on this study is 100 mg BID of oral SCH66336 daily, with 175 mg/m<sup>2</sup> of paclitaxel every 3 weeks. The most common toxicities on this study were myelosuppression and diarrhea. Promising preliminary evidence of efficacy was documented. Six out of 18 evaluable patients achieved a partial response. Notably, 3 of these patients had either progressed on, or after taxane therapy [99]. These clinical results support the preclinical data generated by Shi and co-workers [100].

They demonstrated cytotoxic synergy in vitro and in xenograft models between the combination of SCH66336 and paclitaxel.

#### BMS214662

BMS214662 is a potent inhibitor of human FT *in vitro*, when H-ras and K-ras are used as the farnesyl acceptor substrate. The agent is also a potent inhibitor of soft agar-growth of H-ras and K-ras transformed cells. Unlike the other FTIs, it has been reported that regression of HCT116 xenografts in mice occurred rapidly after treatment with BMS214662, with significant regression seen as early as 24h post treatment, and an 80% regression in 3-4 days [101]. Consistent with these findings, significant apoptosis was seen histologically in tumor tissue, with sparing of normal tissue. In rodents, this agent is active via the oral, intravenous or intraperitoneal route. Phase I studies are ongoing with different intravenous schedules. The single, one hour infusion schedule, repeated every 3 weeks has



been reported in abstract form. In spite of the unconventional dosing schedule for a signal transduction inhibitor, evidence of antitumor activity was observed.

A 40% tumor shrinkage occurred in a patient with non-small cell lung cancer, and prolonged disease stabilization was seen in a patient each with colorectal and pancreatic carcinoma. The most common toxicities were nausea, vomiting and diarrhea. Fatigue, somnolence and anorexia were also observed, but infrequently. The recommended phase II dose on this schedule is expected to be 225 mg/m<sup>2</sup> [102]. Combination studies with standard chemotherapy agents are ongoing, and phase II studies are planned.

The development of a fourth FTI L778123 has been discontinued because of cardiac conduction abnormalities (prolongation of QT interval). At least 2 other FTIs are reported to be commencing phase I clinical trials.

## INTERRUPTION OF SIGNALING PATHWAYS DOWNSTREAM OF RAS

Multiple ras effectors are known, as shown in Fig 2. The most well characterized is the c-raf kinase-MEK-ERK pathway. Conditional MAP kinase activation is important in gene regulation, promoting G1 cell cycle progression before DNA replication, and spindle assembly during both meiotic and mitotic cell division, among other processes. Inappropriate activation of the MAP kinase pathway, through mutations introduced via oncogenes, is a feature of many neoplasms. Most of such mutations are believed to be in ras oncogenes. Several inhibitors of different modules in this pathway are either in clinical trials or are poised to enter trials this year.

### Raf Kinase Inhibitors

#### c-Raf Kinase Antisense Oligonucleotide

ISIS 5132 is a 20-mer phosphothiorate antisense oligonucleotide, designed to hybridize to the 5'-untranslated region of human c-raf kinase mRNA and downregulate the expression of raf kinase. Preclinical studies demonstrated antiproliferative effects in cultured human cell lines with concomitant reduction in c-raf kinase mRNA

[103]. Phase I testing of a continuous intravenous infusion of ISIS 5132 for 21 days every 4 weeks has been reported. The recommended phase II dose was 4 mg/kg/day. At this dose, toxicities were minimal and could not specifically be related to ISIS 5132. Two out of 32 patients had prolonged stabilization of their disease, and one patient with ovarian carcinoma had a significant response with a 97% reduction in CA-125 levels [104]. Phase II studies are underway in prostate, colorectal and ovarian cancer.

### Small Molecular Weight Inhibitors of Raf Kinase

Potent, small molecule inhibitors of c-raf kinase have been described preclinically. These agents inhibited the isolated human enzyme with IC<sub>50</sub>s of 2 – 50 nM. In addition, the proliferation of several human cell lines are potently inhibited *in vitro* and in xenograft models [105]. A member of this class is expected to be introduced into the clinic in Phase I trials this summer.

### MEK Inhibitors

MEK is the most downstream target of ras, for which a clinically useful inhibitor exists. Leopold-Sebolt *et al* have described the pre-clinical properties of PD184322, an orally active highly potent and selective inhibitor of MEK. Tumor growth was inhibited by up to 80% in mice implanted with col 26 and HT 29 colon carcinomas after treatment with PD184322. Efficacy was achieved with a wide range of doses with no signs of toxicity, and correlated with a reduction in the levels of activated ERK in excised tumors. These data indicate that MEK inhibitors represent a promising, non-cytotoxic approach to the interruption of the ras-MAP kinase pathway for cancer therapy [106]. Phase I trials have commenced.

### Inhibition of PI3-Kinase/Protein Kinase B

Phosphoinositide 3'-kinase (PI3-K) is a member of a family of lipid kinases that phosphorylate phosphoinositides [107], and is a downstream effector of ras. Activation of PI3K results in the production of a number of phosphoinositides, which function as second messengers. Wortmannin is a potent inhibitor of

PI3-K, which has no clinical utility, because of toxicity. Active research continues in an effort to identify a clinically useful PI3-K inhibitor. Two downstream targets of PI3-K have been well-characterized. Protein kinase B (PKB; also known as Akt) [108], and p70<sup>S6k</sup> [109]. As discussed above, an unknown farnesylated protein in the PI3-K-Akt pathway is thought to be one of the putative targets of the farnesyltransferase inhibitors.

p70<sup>S6k</sup> mediates phosphorylation and activation of the 40S ribosomal protein S6, which is necessary for cell cycle progression from G<sub>1</sub> into S phase [110-112]. Thus inhibition of p70<sup>S6k</sup> could abrogate the uncontrolled proliferation of malignant cells. A class of novel antibiotics, rapamycin and its analogs inhibit p70<sup>S6k</sup>. Rapamycin (sirolimus) is a macrocyclic lactone, derived from the yeast *Streptomyces hygroscopicus* which is a potent immuno-suppressive agent.

#### CCI-779

CCI-779, the ester of rapamycin, was selected for development as an antineoplastic agent, based on its favorable anti-proliferative profile *in vitro* and *in vivo*. In studies with human tumor cell lines, prostate and breast cell lines were most sensitive (IC<sub>50</sub> <10<sup>-9</sup>M). PDGF-induced proliferation of the human glioblastoma line T98G was also inhibited by CCI-779 (IC<sub>50</sub> = 0.5-2.0 x 10<sup>-12</sup>M). CCI-779 and rapamycin bind to an ubiquitous family of proteins known as immunophilins. The immunophilins are subdivided into cyclophilins and FK-506 binding proteins (FKBP). Rapamycin and CCI-779 bind to FKBP-12 to form a complex that interacts with a novel kinase, termed mammalian target of rapamycin (mTOR, also known as FRAP or RAI1), blocking its activity. This, in turn, results in inhibition of key signal transduction pathways, including those regulated by p70<sup>S6</sup> kinase [113,114] and by phosphorylation of the eukaryotic initiation factor (eIF)-4E-binding protein, PHAS-I (phosphorylated heat- and acid-stable protein) [115-117]. Inhibition of these key signaling pathways results in inefficient translation of mRNAs required for cell-cycle progression through the G<sub>1</sub> phase.

*In vivo* preclinical activity has been documented against prostate, glioblastoma, melanoma, and

colon, breast, and pancreatic carcinoma models in nude mice. Two phase I studies of CCI-779 have been reported.

The first study utilized a 30-minute infusion of CCI-779 administered daily for 5 days with treatment repeated every two weeks. Thrombocytopenia was the most common toxicity. Other toxicities included cutaneous effects, hypocalcemia, hypertriglyceridemia and idiosyncratic allergic-type reactions such as flushing, chest pain and shortness of breath. Objective responses have been seen in non-small cell lung cancer, sarcoma and endometrial carcinoma [118].

In the second study, CCI-779 was administered as a weekly 30-minute infusion. Toxicities were similar to the daily x 5 dosing (myelosuppression, skin toxicity, nail changes, hypertriglyceridemia). Partial responses were documented in 3 patients with renal cell carcinoma, neuroendocrine tumor and breast cancer out of 16 patients evaluable for response [119].

#### INHIBITION OF PROTEIN TRAFFICKING

The heat shock proteins are cellular chaperone proteins, which assist in relocation of proteins from sites of synthesis to sites of action. HSP90 belongs to this family, and participates in multiple signal transduction pathways and is essential for the assembly, folding and activity of several tyrosine kinases and serine-threonine kinases including MAP kinase and raf-kinase [120]. HSP90 is found in all mammalian cell lines. It is part of a multi-protein chaperone system that includes HSP70 and several co-chaperones. An inhibitor of HSP90 is currently undergoing phase I clinical trials.

#### 17-Allylaminogeldanamycin (17-AAG)

Geldanamycin is a member of the benzoquinoid ansamycin class of agents, whose development was terminated because of unacceptable hepatotoxicity [121].

17-Allylaminogeldanamycin (17-AAG), is a derivative of geldanamycin with an acceptable toxicity profile, and was found to be active in breast, melanoma and ovarian mouse xenograft models. 17-AAG binds to the ATP/ADP binding

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site of HSP90 and disrupts its chaperone function. The cochaperone protein p23 binds to the ATP-binding site of HSP90, and inhibition of this binding by geldanamycin has been demonstrated in cultured cells. Another co-chaperone HDJ-2 (HSP40) stimulates the binding of both HSP70 and HSP90 to their client proteins. Levels of HDJ-2 have been shown to increase after geldanamycin treatment in cultured cells. Broad phase I testing of 17-AAG using different treatment schedules are ongoing. Preliminary results are awaited with interest.

**Future Directions**

Ras mutations are one of the most frequent oncogenic events in human tumors. In addition, activation of the ras signaling pathway through upstream events such as HER family receptor over-expression, is also common. Inhibitors of ras signaling cause regression of ras-dependent tumor xenografts. Inhibition of ras signaling pathway molecules is therefore a very promising area of anticancer therapy research. A variety of such inhibitors are now undergoing clinical testing. The utility of this approach will become evident in the next few years.

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